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Zingiber officinale Rosc. modulates gamma radiation-induced conditioned taste aversion

Ashok Sharma [a], Anupum Haksar [a], Raman Chawla [a], Raj Kumar [a], Rajesh Arora [a], Surender Singh [a], Jagdish Prasad [a], F. Islam [b], M.P. Arora [c], Rakesh Kumar Sharma [a,*]

[a] *Division of Radiopharmaceuticals and Radiation Biology, Institute of Nuclear Medicine and Allied Sciences, Brig. SK Mazumdar Road, Delhi 110 054, India*
[b] *Department of Medical Elementology and Toxicology, Jamia Hamdard University, Hamdard Nagar, New Delhi 110062, India*
[c] *Department of Zoology M.M.H. (PG) College, Ghaziabad, C.C.S. University Meerut, U.P., India*

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Abstract

The aim of the present study was to investigate the neurobehavioral protective efficacy of a hydroalcoholic extract of ginger (*Zingiber officinale* Rosc.) in mitigating gamma radiation-induced conditioned taste aversion in Sprague–Dawley rats. Administration of *Zingiber* extract 1 h before 2-Gy gamma irradiation was effective in blocking the saccharin avoidance response for 5 post-treatment observational days, both in a dose- and time-dependent manner, with 200 mg/kg b.w. i.p. being the most effective dose. Highest saccharin intake in all the groups was observed on the fifth post-treatment day. The potential of ginger extract to inhibit lipid peroxidation induced by radiation (2 Gy) and ascorbate-ion stress in brain homogenate and its ability to scavenge highly reactive superoxide anions were evaluated. The 1000-μg/ml and 2000-μg/ml concentration of ginger extract showed the highest efficiency in scavenging free radicals and in inhibiting lipid peroxidation. The lipid peroxidation and superoxide-anion scavenging ability of the extract further supports its radioprotective properties. The results clearly establish the neurobehavioral efficacy of ginger extract and the antioxidant properties appear to be a contributing factor in its overall ability to modulate radiation-induced conditioned taste aversion. Ginger extract has tremendous potential for clinical applications in mitigation of radiation-induced emesis in humans.

Keywords: Conditioned-taste aversion; Emesis; Lipid peroxidation; Neurobehavioral; Radioprotection; *Zingiber officinale*

1. Introduction

Ionizing radiation is known to have significant effects on a variety of neurobehavioral factors (Harding, 1988), of which the consumption behavior is relatively radiosensitive (Hulse and Mizon, 1967). Exposure to ionizing radiation is known to bring about changes in food preference, reduce food and water consumption (Goel et al., 1997; Milgram and Krames, 1977) and also to produce nausea and vomiting (Garcia et al., 1955). Exposure to even a few Gray of radiation results in emesis in humans, the response being dose dependent up to 10 Gy, beyond which the incidence of emesis decreases (Mattsson and Yochmowitz, 1980). Emesis can result in severe performance decrement of personnel operating in high radiation environments and causes great inconvenience to patients undergoing radiotherapy (Walter, 1987).

As emesis is not observed in rodents, conditioned taste aversion (CTA), the most reliable and radiosensitive form of neurobehavioral conditioning, is taken as an equivalent manifestation (Shobi and Goel, 2001) and used as a model for radiation-induced gastrointestinal distress and emesis (Lushbaugh, 1947). It is possible to produce such conditioned aversions by pairing a novel tasting solution with a toxin, e.g., a single whole-body exposure to ionizing radiation, so that when the solution is presented later, further consumption is avoided (Garcia et al., 1955, 1985). This avoidance behavior is rapidly acquired after a single

* Corresponding author. Tel.: +91 11 23984480; fax: +91 11 23919509.
E-mail address: rks@inmas.org (R. Kumar Sharma).

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pairing of the solution with the toxin and is found to be long lasting (Carrie et al., 2001).

A number of studies have shown that radiation-induced CTA/emesis operates through various pathways depending on the species (Rabin and Hunt, 1986, 1992), the radiation dose (Rabin et al., 1998), the time course (Smith and Carrol, 1974) and the body part, which is exposed (Smith et al., 1981). Radiation acts on the abdominal region (stomach+intestine), resulting in the release of serotonin, from where the splanchnic and vagal afferents through 5HT3 and 5HT5 receptors (Endo et al., 2000) activate the vomiting center in brain (Barnes, 1984). In addition, radiation can directly affect neural activity in brain and also activate certain sensory receptors (Neuronal hypothesis) (Rabin et al., 1984). It has also been postulated that radiation can lead to production of certain biological factors, like histamines (Bhargava and Dixit, 1968) or nitric oxide (Wegener et al., 2001) or certain peptides like VIP (Carpenter et al., 1984), angiotensin, neurotensin, vasopressin (Wu et al., 1985), YY (Harding et al., 1985), endogenous opiates (Mickley et al., 1983), ACTH (Hennessy et al., 1980) and prostaglandins (Carpenter et al., 1988), which reach the chemoreceptor trigger zone (CTZ) in Area Prostema (Humoral hypothesis), through blood and CSF leading to the activation of the vomiting center (Hunt and Kimeldorf, 1967). Summarizing all the available information, it can be concluded that there are at least three ways in which radiation can trigger a CTA/emetic response; through the gastrointestinal tract, via the nervous system (neuronal hypothesis) and through the humoral system (Humoral hypothesis), ultimately leading to the formation of long-term CTA memory.

A number of synthetic radioprotective drugs like WR-2721, WR-1607, Hoechst-33258, Hoechst-33342, glucan, etc. have been evaluated for radioprotection (Giambarresi and Jacobs, 1987), but the toxicity associated with the effective doses severely constrains their applicability, specifically for behavioral radioprotection and clinical radiotherapy. For example, WR-1607, although maintains performance (continuous-avoidance task and visual-discrimination task) (Sharp et al., 1968), causes intense nausea and vomiting (Turbyfil et al., 1972), while WR-2721 is known to have significant behavioral toxicity and induces performance degradation (Bogo et al., 1985). In view of this, attention now has been shifted towards evaluation of plant products as radioprotectors due to their effectiveness and low toxicity (Arora et al., 2005). A number of plants like *Ocimum sanctum* (Uma Devi and Ganasoundari, 1995), *Phyllanthus niruri* (Uma Devi et al., 2000), *Podophyllum hexandrum* (Goel et al., 1999), and *Ginkgo biloba* (Emerit et al., 1995a), have been found effective in providing protection against radiation-induced lethality, chromosomal aberrations, micronuclei formation, and generation of free radicals, GI-syndrome. However, relatively few studies have focused on mitigation of neurobehavioral toxicity induced by radiation (Arora et al., 2005).

Fresh/dried rhizomes of the ginger plant *Zingiber officinale* are widely used as a spice and flavoring agent world-over. Ginger rhizomes have also been widely recommended as a remedy for nausea, vomiting, gastrointestinal symptoms and to mitigate chemotherapy-induced emesis in various systems of medicine (Tanaka, 1994; Flake et al., 2004). Its active constituents, β-sesquiphellendrene, β-bisabolene, ar-curcumene, 6-shogaol, zingiberene, terpenoid, 6-gingerol and 6-gingesulfonic acid have been reported to inhibit experimentally induced gastric ulcerations in rats (Yamahara et al., 1988). Ginger extract has been found to be neuroprotective in various behavioral tasks, e.g., maze learning (Topic et al., 2002a,b), and inhibitory avoidance learning (Topic et al., 2002a,b). Ginger (*Z. officinale* Rosc.) is a potent antioxidant (Aeschboch et al., 1994) and has been reported to render whole-body radioprotection under in vivo conditions (Jagetia et al., 2003).

The present study was therefore undertaken to evaluate the neurobehavioral radioprotective efficacy of ginger extract (*Z. officinale* Rosc.) against radiation-induced CTA.

2. Materials and methods

2.1. Animals

Sprague–Dawley adult male rats (12–15 weeks old; 323±25 g) that were in-bred at the Animal House of the Institute of Nuclear Medicine and Allied Sciences, Delhi were used for the experiments. Animals were kept under standard laboratory conditions with photoperiod of 12 h/day and temperature of 25±2 °C. The rats were housed individually in polyvinyl cage and fed standard animal food pellets (Golden Feeds, Delhi, India) and were offered tap water ad libitum. All the procedures were carried out in strict compliance with the Animal Ethics Committee rules and regulations followed in this Institute.

2.2. Irradiation

Each rat was placed in a wire gauze container and put in the Co^{60} Gamma irradiator (Model 220, Atomic Energy Commission, Canada), having a dose rate of 37.2 rads/min. and exposed to 2 Gy of radiation, during the course of the study. Dosimetry was carried out with Baldwin Farmer secondary dosimeter and Fricke dosimeter.

2.3. Drug

Ginger (*Z. officinale*) rhizomes were collected during Dec–Feb 2003. The plant material was identified by Dr.

Rajesh Arora, Medicinal Plant Scientist, at the Institute of Nuclear Medicine and Allied Sciences, (INMAS), Delhi. A voucher specimen (No. INMAS/*Zingiber officinale*/2003/RPG-2003) has been deposited in the repository at INMAS, Delhi, India. The rhizomes were crushed, extracted in 50% ethanol at room temperature for 72 h, filtered, concentrated in a rotary evaporator (Buchi, Switzerland) at 50±5 °C, lyophilized (FTS Systems, USA) and stored at −70 °C in a deep freezer (New Brunswick, USA) until use. The yield on w/w basis was approximately 9±1%. The extract was suspended in 10% ethanol and filtered through Millipore 0.2-μm filter immediately prior to use. This extract was administered intraperitoneally to experimental animals 1 h before exposure to radiation (2 Gy). For in vitro studies, a minimum of 5% ethanol was used as a vehicle.

2.4. Conditioning procedure

All animals were habituated to the laboratory conditions at least 4 weeks prior to training. Rats were trained for 23.5 h water deprivation schedule for 10 days (conditioning period), wherein each animal was offered sterilized tap water only, i.e., one bottle paradigm for 30 min during affixed time everyday (10:00–10:30 a.m.) (Moron and Ballesteros, 2002). Water consumption of the individual animal per day was recorded. On the 10th day of conditioning period, all the rats were given a choice between 0.1% saccharin solution and tap water for 30 min (two-bottle regime) (Miranda and Hong, 2001), and their respective intake of 0.1% saccharin solution and tap water was recorded. Only those animals, which exhibited saccharin solution intake of more than 50% of their total fluid intake, were selected for participation in the experiment. Body weight of the animals was recorded daily before beginning the experiments.

Immediately following the conditioning session, the rats were divided into the following groups:

Group I Vehicle + Sham Radiation
Group II Vehicle + Radiation
Group III Ginger extract + Sham Radiation
Group IV Ginger extract + Radiation

Homogenous groups were planned so that animals in a group had comparable levels of saccharin intake, showing minimum or no variation.

After this, according to the groups, the animals ($n=24$) were injected with vehicle (10% ethanol, i.p.) or ginger extract (i.p.) and, after 1 h, were Sham irradiated or delivered 2 Gy of Gamma irradiation from a Co^{60} gamma source.

Twenty-four hours after the experiment, the animals were again given the choice between saccharin solution and tap water, and their respective intake was recorded. This procedure was repeated for 5 post-irradiation days (Mukherjee et al., 1997).

The experimental procedure was used for testing four different drug concentrations, i.e., 50, 100, 150 and 200 mg/kg body weight, with six animals per group.

2.5. Superoxide ion scavenging activity

The superoxide ion quenching ability of the ginger extract was determined using the method of Kakkar et al. (1984). Varied concentrations of plant extract were mixed with 1 ml of sodium pyrophosphate buffer (0.052 M, pH 8.3) and 0.1 ml of phenazine methnosulfate (186 μM). 300 μl of nitrobluetetrazolium (300 μM) was added to the above solution and the final volume adjusted to 3 ml. Thereafter, the reaction was initiated by adding 200 μl of NADH (780μM). The whole solution was then incubated at 37 °C for 90 s. The reaction was terminated by the addition of 1 ml of glacial acetic acid. The resultant mixture was shaken with 4 ml of *n*-butanol layer and allowed to stand for 10 min at room temperature. The *n*-butanol layer was separated by centrifugation and the color intensity of the chromogen in the *n*-butanol layer was measured at 560 nm against *n*-butanol.

2.6. Preparation of whole-brain homogenate

Rats were randomly selected and sacrificed by cervical dislocation and dissected. The whole brain was taken out, and 10% homogenate was prepared in cold-buffered saline (pH 7.4) using Potter Elvejam homogenizer and filtered to get a clear homogenate.

2.7. Estimation of Iron/Ascorbate and radiation (2 Gy)-induced lipid peroxidation

The method of Srour et al. (2000) was adopted for estimation of lipid peroxidation. 2 ml of 10% whole brain homogenate was taken in a series of 35-mm Petri dishes and the desired amounts of different fractions were added and mixed gently to form a homogenous solution. Lipid peroxidation was initiated by adding 20 μl of ferric chloride (0.5 mM) with 200 μl of ascorbate (1 mM) and also by exposure to radiation (2 Gy). Thereafter, the petri dishes were incubated at 37 °C for 30 min. One milliliter of homogenate was pipetted out for estimating lipid peroxidation levels in terms of thiobarbituric acid-reactive substances (TBARS), measured by recording the absorbance at 535 nm in a spectrophotometer (Varian Instruments, USA). Lipid peroxidation value, calculated as follows, is expressed as nanomoles of malonidialdehyde formed per hour per gram of tissue.

$$\text{nm of MDA formed/h/g of tissue} = \text{O.D.}\ (535\text{nm}) \times \text{dilution factor} / \text{molar extinction coefficient}_{\text{MDA:TBA complex}} \times \left(1.56 \times 10^6\right)$$

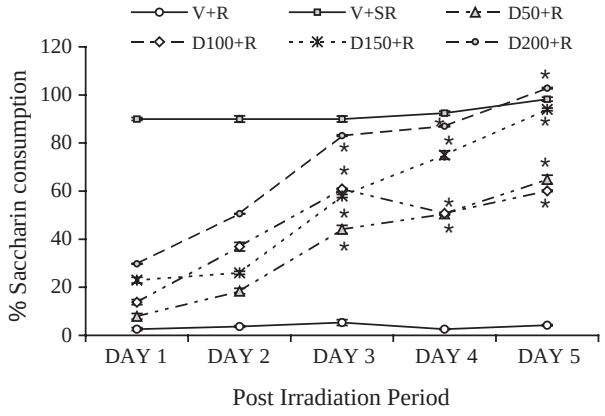

Fig. 1. Effect of different doses of ginger extract on conditioned taste aversion over 5 days. Values are expressed as mean±S.D. [V=vehicle; R=radiation (2Gy); SR=Sham radiation; D=dose of ginger extract (mg/kg b.w.)] *$p<0.05$; *df*: 4,20 of % of saccharin intake (between the days viz., days 3, 4 and 5.

2.8. Statistical analysis

In the above experimental design, the extinction of radiation (2 Gy)-induced CTA was studied. The analysis was done using Radiation × Ginger extract × Days, i.e., $2 \times 2 \times 5$ mixed ANOVA, which was performed repeatedly for each drug dosage given to the rats. Two-way analysis of variance (ANOVA) was used to determine the statistical significance of treatments on saccharin intake (i.e., CTA) or water. Student's *t*-test was used to determine effect of drug on biochemical estimations and inherent bioactivity of the drug. Differences were considered significant when $p<0.05$.

3. Results

3.1. Effect of Zingiber extract on basal saccharin intake

As shown in Fig. 1, ginger extract was observed to considerably restore saccharin consumption of animals given 2 Gy of radiation, as compared to the radiation control group where animals did not receive the ginger extract. The intake of saccharin was highly enhanced, reaching 5% level of significance ($p<0.05$) with the dose of 200 mg/kg b.w. The saccharin consumption restoring effect was found to increase in a dose-dependent manner, with 200 mg/kg b.w. as the most effective extract concentration, where the maximum saccharin intake was achieved on the fifth post-irradiation day. The analysis of percentage intake of saccharin in the group administered 50 mg/kg b.w. ginger extract revealed significant main effects of treatment of radiation ($F_{1,16}=158.24$; $P<0.001$) and ginger extract administration ($F_{1,16}=11.95$; $P<0.05$), revealing that administration of ginger extract significantly reduces the effect of radiation-induced CTA over a period of 5 days, while the interaction between the two was not found to be

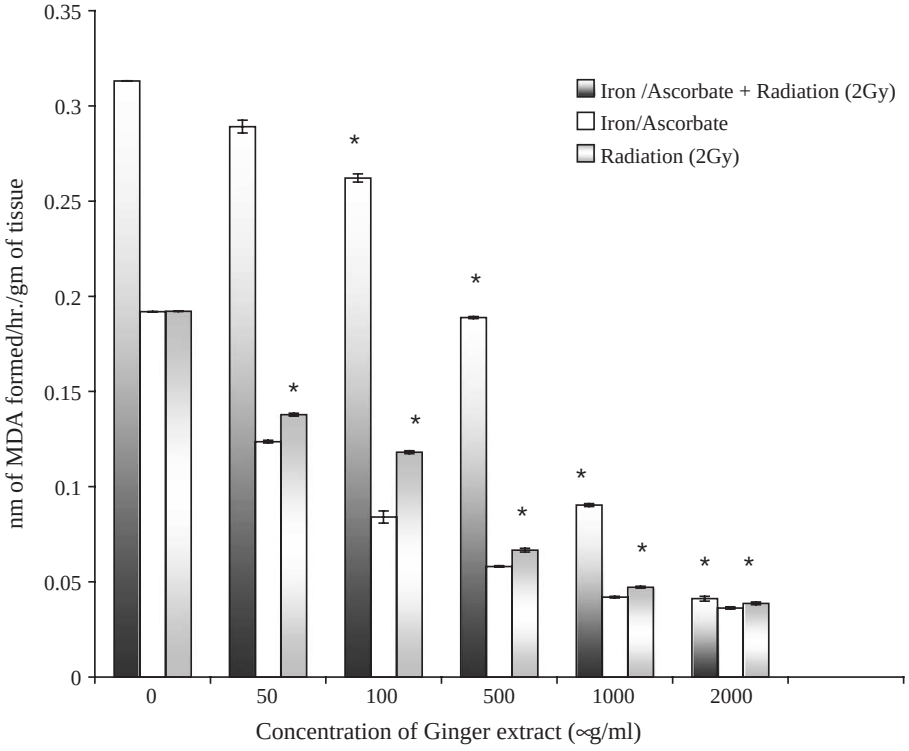

Fig. 2. Effect of different doses of ginger extract (50–2000 μg/ml) on iron/ascorbate, radiation (2 Gy) and iron/ascorbate+radiation (2 Gy)-induced lipid peroxidation [activity was expressed as nanomoles of MDA formed per hour per gram of brain tissue. All the samples are taken in triplicates and values are expressed as mean±S.D. * Drug+radiation vs. untreated control (0% inhibition); $p<0.05$.

significant at $P<0.05$, implying that there is no effect of radiation exposure on the ginger extract administration, i.e., the two events are independent. A similar effect was observed in the group administered 100 mg/kg b.w. ginger extract, revealing significant main effects of radiation exposure ($F_{1,16}=5.339$; $P<0.05$) and treatment of ginger extract ($F_{1,16}=42.75$; $P<0.01$), while the interaction effect between the two was insignificant ($P<0.05$). In a separate analysis of percentage intake of saccharin, the group administered 150 mg/kg b.w. dosage exhibited significant main effects of radiation exposure ($F_{1,16}=14.83$; $P<0.001$) and ginger extract administration ($F_{1,16}=52.87$; $P<0.01$), although the interaction between the two was found to be insignificant ($P<0.05$). Similarly, in the group administered 200 mg/kg b.w., significant main effects of radiation and drug treatment (i.e., $F_{1,16}= 47.28$; $P<0.05$ and $F_{1,16}= 27.96$; $P<0.05$) respectively were observed while the interaction between the two was insignificant ($P<0.05$). In a separate analysis aimed at evaluating the effect of administration of ginger extract (over the 5 days) on the percentage of saccharin intake by the rats pre-exposed to 2 Gy, two-way ANOVA was used and the effect of ginger revealed maximal extinction of CTA on the third, fourth and fifth day (*df* 4,20) as represented in Fig. 1.

3.2. Inhibition of Lipid peroxidation

Anti-lipid peroxidation studies were performed on rat brain tissue homogenate using the TBARS method. The percent inhibition of radiation (2 Gy)-induced lipid peroxidation by ginger extract (2000 μg/ml; 79.85%) was found to be significant ($p<0.05$) as compared to the control (0% inhibition). In case of the lipid peroxidation induced by iron and ascorbate (treated as positive control), a similar pattern, i.e., decrease in malondialdehyde (MDA) formation with increase in dose-dependent manner, was observed and the maximum % inhibition of lipid peroxidation activity was observed at 2000 μg/ml (81.095%). The % inhibition of lipid peroxidation activity induced by the combined stress of iron/ascorbate and radiation (2 Gy) was found to be highly significant (86.85%; $p<0.05$) as compared to control (0% inhibition) (Fig. 2).

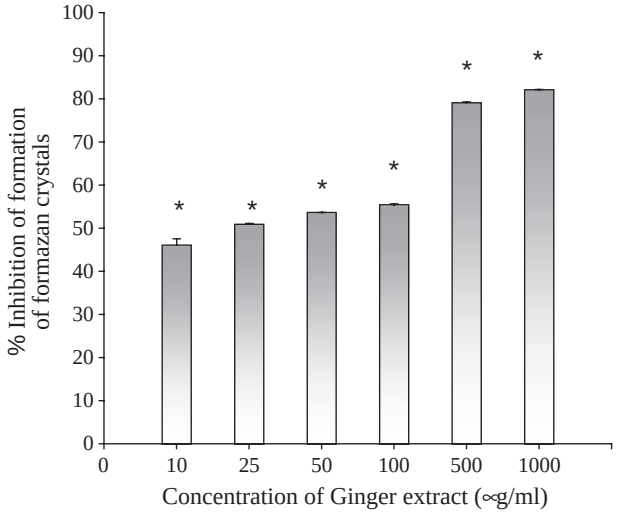

Fig. 3. Evaluation of superoxide-ion scavenging ability of the extract expressed as percent inhibition of formation of formazan crystals vs. various concentrations of ginger extract. *Drug treated vs. control (0% inhibition); $p<0.05$.

3.3. Free radical scavenging potential

Free radical scavenging capacity was studied using nitroblue tetrazolium as a marker substrate and the percent inhibition of formazan crystal was taken as the underlying basis of the potential of the ginger extract to scavenge the free radicals (Kakkar et al., 1984). The percent inhibition of formazan crystal formation was found to be increasing in a dose-dependent manner and maximum activity was observed at 1000 μg/ml (82.12%), which was found to be significant ($p<0.05$) at all doses of ginger extract as compared to control (no plant extract: 0% inhibition) (Fig. 3).

4. Discussion

The present study reports the neurobehavioral radioprotective efficacy of ginger extract against radiation-induced CTA. Radiation-induced CTA has been observed in cancer patients undergoing radiotherapy (Smith et al., 1984) and is known to induce changes in the patient's choice of food and related nutritional needs for warranting the search for effective agents to mitigate radiation-induced emesis in humans. The intestine is highly radiosensitive and post-irradiation release of serotonin (5-HT) from the gastric enterochromaffin cells could manifest nausea/emesis in humans and taste aversion in rats (Lopez et al., 1999). Moreover, 5-HT also affects food intake both in humans and animals (Garcia et al., 1956; Middleton and Young, 1975). Ginger extract provided significant protection ($p<0.05$) against 2-Gy gamma radiation-induced CTA in male rats (Fig. 1) in a dose- and time-dependent manner up to 200 mg/kg body weight in the current study. A concentration of 200 mg/kg body weight was found to be most effective in extinguishing radiation-induced taste aversion and completely restored the normal taste preference by the fifth post-irradiation day.

There are several ways to examine the aversive properties of a substance using a CTA paradigm. One way is to look at the acquisition of CTA and another is to examine the extinction of CTA. In the present study, the extinction of CTA was examined. Animals exposed to 2-Gy gamma radiation alone exhibited a lower consumption of saccharin solution in comparison to untreated animals (sham radiation group) for a period of 5 post-irradiation

days, indicating no extinction of aversion in this group. However, pre-irradiation administration of ginger extract extinguished the aversion over five post-irradiation trials, such that by the fifth day, they consumed saccharin at comparable levels to untreated group. Ginger extract provided significant protection against 2-Gy-induced CTA, between 3rd and 5th post-irradiation days. It can be concluded from the results of the present study that ginger extract helped in the extinction of CTA and not in the acquisition of aversive information.

In order to evaluate the underlying mechanism of this protective behavior, free radical scavenging ability and anti-lipid peroxidation potential were also evaluated. Treatment with ginger extract in a dose range of 50 μg/ml to 2000 μg/ml resulted in a twofold decrease in MDA formation in brain homogenate exposed either to radiation (2 Gy) or iron/ascorbate stress. On the other hand, exposure to combined stress (2Gy+iron/ascorbate), resulted in a significant decrease (~11-fold) in MDA formation in brain tissue that was significant when compared to treated and untreated control ($p<0.05$).

Ginger extract was found to have immense antioxidant potential to scavenge free radicals, possibly due to its bioactive constituents, with the ability to donate electron(s) and scavenging the free radicals, specifically superoxide anions. This characteristic of plant extract was found to have a positive correlation with its anti-lipid peroxidation potential. This indicated that ginger extract acts as an antioxidant and can protect the brain tissue against radiation-induced damage by maintaining the integrity of cell membranes. Similar reports have been reported earlier by Lu and Lai (2003) using ginger oil.

The neurobehavioral effect of ginger extract on radiation-induced CTA can be attributed to the above-mentioned properties. In addition, the 5-HT antagonistic action of *Z. officinale* (Hasenohrl, 1998) negates the serotonin released in the intestine, restoring gastrointestinal integrity and blocking the visceral pathway (Huang et al., 1991; Yamahara et al., 1989; Sharma and Gupta, 1998), thereby counteracting the 5-HT mediated suppression of fluid intake and development of aversion for saccharin (Holtman et al., 1989). Ginger extract, through its neuromodulatory effect, may be acting at the brain level protecting it from radiation-induced activation of sensory receptors and mitigating the disturbances in neural activity (Hasenohrl et al., 1996). Its action could also be mediated via suppression of nitric oxide production (Ippoushi, 2003), or by virtue of its anti-cyclooxygenase activity, it may be acting at the humoral level, suppressing inflammatory prostaglandins (Shen et al., 2003).

The present study demonstrates that ginger extract offers good neurobehavioral radioprotection against radiation-induced CTA in rats. Further studies are necessary to identify the bioactive constituents in ginger extract (*Z. officinale*) that are responsible for neurobehavioral radioprotection against CTA.

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